Supplemental Figure S1

(A) Immunofluorescence using anti-MITF antibody of IGR37 cells grown in DMEM, MEM or MEM supplemented with 4 mM glutamine

(B) Western blot of IGR37 cells grown in MEM supplemented with serine and glycine with or without glutamine. U0126 (10 μM) was added where indicated.

(C) Western blot of IGR37 cells grown in DMEM or MEM supplemented with serine and glycine without glutamine in the presence of absence of 1 μM of the calcium-dependent kinase inhibitors calmidazolium or KN-93.

(D) qRTPCR of MITF mRNA expression relative to GAPDH mRNA of IGR37 cells grown in the presence or absence of 1 μM calmidazolium for indicated times after glutamine deprivation. Results expressed as average ± SD of 3 biological replicates.

Supplemental Figure S2

(A) Heatmap of gene array data from IGR37 cells grow in in DMEM or MEM (See also Supplemental Table 1).

(B) Heatmap of MITF-bound genes (See also Supplemental Table S2).

(C) qRT PCR using *ATF4*-specific primers of mRNA from IGR37 cells grown in DMEM or MEM performed in triplicate with levels normalized to *GAPDH*. Results expressed as average ± SD.

(D) Triplicate luciferase assays from cells transfected with an ATF4-promoter-luciferase reporter co-transfected with an MITF-expression vector. Results expressed as average ± SD.

(E) UCSC browser screenshot showing biological replicates of MITF binding to the ATF4 promoter detected using ChIP-seq.

(F) Immunofluorescence of IGR37 cells grown in DMEM or MEM supplemented with serine and glycine (-Gln). Quantification of mean fluorescence intensity is shown in right panel with results expressed as average ± SD. Scale bar =10 μM.

(G) Western blot of IGR37 cells grown in DMEM or MEM supplemented with serine, glycine or glutamine for 24 hr or 10 μM thapisgargin for 4 hr.

(H) Western blot of 501mel cells grown in DMEM or MEM supplemented with serine and glycine and indicated concentrations of glutamine for 24 hr.

(I) Western blot using indicated antibodies of IGR37 cells grown in DMEM or MEM supplemented with serine, glycine and 0.2 mM glutamine for indicated times.

(J) Heatmap from gene array data from IGR37 cells showing clustering of ATF4 target genes identified by ChIP-seq (See also Supplemental Table S3).

(K) Immunofluorescence of indicated cell lines expressing doxycycline-inducible ATF4 grown +/- 100 ng doxycycline for 24 hr. Scale bar =10 μM.

(L) SDS PAGE analysis of IGR37 cells ^{35}S-methionine pulse labeled for 20' cultured under indicated conditions. Total radiolabelled protein is shown in the left panel together immunoprecipitated MITF. Coomassie stained protein is shown on the right.

Supplemental Figure S3

(A) Senescence-associated β-gal activity in cell lines grown in DMEM and depleted for MITF, or in MEM or MEM supplemented with serine (S), glycine (G) or glutamine (Q). Scale bars =10 μM.

(B) Correlations in cell lines between the GSS and Verfaillie invasiveness signature, or invasiveness (right panel).

(C) Quantitative RT PCR from IGR37 cells grown in DMEM or MEM supplemented with serine and glycine (-Gln). Results expressed as mean ± SD of at least 3 biological replicates.

Supplemental Figure S4

(A,B) Western blot of indicated cell lines grown in DMEM and treated with of Salubrinal (20 μM)

(C) Cell death assays of indicated melanoma cell lines grown without glutamine for 48 or 72 hr, or for 72 hr in the presence of ISRIB (10 μM) or Salubrinal (20 μM) as indicated.

(D) Western blot using indicated antibodies of extracts from cells treated over time with nelfinavir (20 μM).

(E) Western blot of melanoma cell lines.

(F) Quantitative RT PCR for indicated genes of mRNA from 501mel or SKmel28 cells grown in DMEM inducibly expressing ATF4 in response to 100 ng doxycycline. Error bars represent mean +/- S.D of at least 3 biological replicates.

Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma

Paola Falletta[1,10], Luis Sanchez-del-Campo[1,10], Jagat Chauhan[1], Maike Effern[2], Amy Kenyon[3], Christopher J Kershaw[4], Robert Siddaway[1], Richard Lisle[1], Rasmus Freter[1], Matthew J Daniels[5], Xin Lu[1], Thomas Tüting[6], Mark Middleton[7], Francesca M Buffa[7], Anne E Willis[8], Graham Pavitt[4], Ze'ev A Ronai[9], Tatjiana Sauka-Spengler[3], Michael Hölzel[2] and Colin R Goding[1]*

Supplemental Information

Supplementary Methods

Antibodies

Antibody	Source	Catalogue number
MITF (Rabbit polyclonal)	Home made	-
MITF (Mouse monoclonal)	Home-made	-
PAX3 (Mouse monoclonal)	DSHB Iowa	-
BRN2 (Mouse monoclonal)	Home-made	-
ERK (Rabbit polyclonal)	SantaCruz	SC-154
Actin (Mouse monoclonal)	Sigma	A4700
CREB (Rabbit polyclonal)	Cell Signaling	9197
pCREB (Rabbit polyclonal)	Cell Signaling	9198
E-cadherin (Mouse monoclonal)	BD Bioscience	610182
p-eIF2α (Ser51) (Rabbit monoclonal)	AbCam	Ab32157
PARP	Cell Signalling	9452
ATF4 (Rabbit polyclonal)	SantaCruz	SC-200
Fibronectin (Mouse monoclonal)	BD Bioscience	610078
N-cadherin (Mouse monoclonal)	BD Bioscience	610921
AXL (Rabbit polyclonal)	Cell Signaling	8661
Flag M2 (Mouse monoclonal)	Sigma	F1804

Chemicals

Calmidazolium, KN-93, ISRIB, U0126 and Doxocycline were from Sigma, Human TNFα from Peprotech, and Salubrinal was from Calbiochem.

RT PCR Primers

Gene	Forward	Reverse
MITF	5'-AAACCCCACCAAGTACCACA-3'	5'-ACATGGCAAGCTCAGGAC-3'
GAPDH	5'-CCCACTCCTCCACCTTTGA-3'	5'-CATACCAGGAAATGAGCTTGACAA-3'
β-Actin	5'-AGAAAATCTGCACCACACC-3'	5'-GGGGTGTTGAAGGTCTCAAA-3'

ATF4	5'-TCAAACCTCATGGGTTCTCC-3'	5'-GTGTCATCCAACGTGGTCAG-3'
CDH1 (E-cadherin)	5'ATTCTGATTCTGCTGCTCTTG-3'	5'-AGTAGTCATAGTCCTGGTCTT-3'
CDH2 (N-cadherin)	5'-TGTTTGACTATGAAGGCAGTGG-3'	5'-TCAGTCATCACCTCCACCAT-3'
ZEB1	5'-ACCCTTGAAAGTGATCCAGC-3'	5'-CATTCCATTTTCTGTCTTCCGC-3'
ZEB2	5'-TCCAGAAAAGCAGTTCCCTTC-3'	5'-CACACTGATAGGGCTTCTCG-3'
FN1(Fibronectin)	5'-GGAAAGTGTCCCTATCTCTGATACC-3'	5'-AATGTTGGTGAATCGCAGGT-3'
TWIST	5'-CGGGAGTCCGCAGTCTTA-3'	5'-TGAATCTTGCTCAGCTTGTC-3'
AXL	5'-GTGGGCAACCCAGGGAATATC -3'	5'-GAGCTGGCTGACCACTATCC-3'

Table S1. Gene array data showing up- and down-regulation of genes in IGR37 cells in response to culture over time in MEM related to Supplemental Fig. 2A

Table S2. MITF bound target genes in each group shown in Supplemental Fig. 2B

Table S3. ATF4 target genes by group highlighted in Supplemental Fig. 2J

Table S4. Genes comprising the gene expression signatures used throughout this study.

Table S5. Gene array data showing up- and down-regulation of genes in 501mel cells in response to treatment with salubrinal